

# PHYSIOLOGICAL STUDIES ON HIBERNATION IN THE POTATO BEETLE, *LEPTINOTARSA* *DECEMLINEATA* SAY.<sup>1</sup>

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## INTRODUCTION.

An inquiry into the nature and cause of the phenomenon of hibernation has for many years engaged the attention of investigators. The literature on the physiological study of hibernating mammals covers a wide range of research, the most significant of which appears to be into the effects of temperature, food and gaseous exchange. The experimental evidence brought forward by Dubois (10), Pembrey (17), Valentin (25), Weinland and Riehl (29) and others, indicates that hibernating animals placed

<sup>1</sup> A thesis in zoölogy presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

in the presence of an abundance of food and warmth remain impassive. This has led to the conclusion that hibernation is some inherent, deep seated phenomenon. Recent studies by Carlier (8), Rasmussen (18), Sheldon (22) and others, upon the nature and function of the so-called hibernating gland, show it widely present in mammals.

The literature on physiological studies of hibernating insects is not so extensive. Baumberger (1), Bodine (3), Breitenbecher (6), Roubaud (19), Sanderson (20), Tower (24) and others, however, have investigated problems of temperature, moisture, and gaseous exchange as they relate to insects. According to Baumberger (2), hibernation has resulted from the repeated effect of winter upon the species and the rhythmical phenomenon has been determined by the habits of the insect. Roubaud (19) recently advanced the view that a lowered metabolic activity permits a progressive physiological purification. According to this author, two factors, *anhydrobiosis*, and *athermobiosis* (dehydration and absence of heat), in themselves inhibitory, become the unexpected factors of reactivation by favoring the processes of excretion during dormancy.

Most investigators whether dealing with hibernation in vertebrates or invertebrates agree that the influence of temperature in the process is subordinated, and that hibernation is preceded usually by a period of extensive feeding and a consequent reduced metabolic activity. It is also well known that food is an important factor in influencing the habits of animals. The manifold instances of food storage indicate a varying adaptability, the most significant being the storage within the body of the animal of reserve nutrients in the form of fat.

With insects the phenomenon of hibernation is unique, because it may occur in any stage of the life cycle of different insects, from the egg to the adult. It seemed advisable, therefore, to investigate the problem of nutrient storage in insects and to determine the specific mode of its utilization during the progress of hibernation.

This contribution presents the results obtained from physiological studies on the potato beetle, *Leptinotarsa decemlineata* Say, before, during, and after hibernation.

The author desires to express his appreciation to Prof. C. E. McClung, and other members of the Zoölogical Department of the University of Pennsylvania, for generous aid and suggestions. To Doctor J. H. Bodine he is deeply indebted for helpful criticism during the progress of the work and for advising investigation of this problem.

#### MATERIAL AND METHODS.

The adult potato beetle, *Leptinotarsa decemlineata* Say, used exclusively in these investigations, was reared from eggs deposited by overwintering adults.

The methods adopted for different lines of investigation varied. The type of cage for determining the effect of various food plants consisted of ordinary six-inch flower pots filled with soil, in the center of which was sunk a 5 x 1 inch glass vial containing water and fresh food plant. The covering to this cage was made of mosquito wire in the form of a circular cage of a diameter to fit snugly within the inner upper rim of the flower pot, and varying in height from 12 to 18 inches. These cages were set in troughs of soil in an outdoor breeding house. A more elaborate cage consisted of a wooden framework (20 x 18 x 14 inches), constructed of inch pine, enclosed with mosquito wire and set on



FIG. 1. Types of cages used in the food experiments. This figure shows two types: (1) circular cages composed of flower pots sunk in the soil with mosquito wire tops; (2) large oblong cages constructed of inch pine, covered with mosquito wire and set on the surface of the soil. Foliage was kept fresh in the cages by inserting them in vials of water.

the surface of the soil in the breeding house or in the field (Fig. 1). In all cages it was impossible for beetles to escape or for others to find entrance from the exterior. Access for the worker was by a convenient opening on the top or side of the cage.

The water content was obtained by first weighing and slitting the animals and afterwards placing them in an oven at a temperature between 90–95° C., until a constant weight was obtained. The percentage of water was computed on the basis of dry weight.

Although the extraction of fat from the body of an organism with solvents cannot be achieved quantitatively without change in the nature of the compounds in which the fatty acids are present, this was not considered an objection in these experiments as the result desired was the total fat content in whatever form it might occur. The tissues, however, had to be dried and powdered. To prevent partial oxidation of the more unstable unsaturated acids, the drying took place at room temperature, or in an oven at a temperature not exceeding 30° C. The extracting solvent used extensively was a mixture of equal proportions of ether and alcohol, which was warmed and renewed several times by decantation. In addition, the method described by Voltz (28), and modified to suit the material under investigation was also used. Calculations were made in percentage based on dry weight of the organism.

Metabolism determinations (respiratory exchange) were obtained by methods essentially similar to those described by the author (12) in a previous publication. Other methods used will be described in the text under appropriate headings.

## EXPERIMENTS AND DISCUSSION.

### *Food Experiments.*

The effect of different food plants on potato beetles of the first generation emerging from pupation was studied. Altogether, during June, 1923, and 1924, about 800 beetles were divided into four main groups and fed on various food plants as follows:

- Group 1. The foliage of potato, tomato, eggplant and peppers.
- Group 2. The tubers or fruit of the above plants.
- Group 3. The foliage of potato for six days, followed by sliced



non-solanaceous foods like beets, carrots, squash and cucumbers.

Group 4. Non-solanaceous foods exclusively.

*Number of Days Feeding Before<sup>1</sup> Hibernation.*

The number of days feeding on different food plants before hibernation took place is shown in Tables I.-IV., and in Fig. 2. Group 1, fed on foliage required an average of 16 days, whereas group 2, fed on tubers and fruit required an average of 13 days. Evidently, to accumulate sufficient nutrients necessitated a more extensive feeding on foliage than on tuber or fruit. The experiment with group 3, fed on potato foliage for six days followed by a period of feeding on sliced non-solanaceous foods, resulted in, first, an increase in the number of feeding days essential for nutrient storage to an average of 23.6 days; second, a mortality of 23 per cent.; third, unsuccessful hibernation of 32 per cent. (which died during the process).

TABLE I.

NUMBER OF DAYS FOR BEETLES FED ON FOLIAGE TO ENTER HIBERNATION.

Potato.		Tomato.		Eggplant.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
75.....	14	100.....	15	80.....	11
25.....	21			20.....	20
Average.....	17.5				15.5

*Cages Receiving No Water.*

80.....	11	10.....	11	90.....	18
10.....	13	80.....	13	10.....	20
10.....	Died	10.....	Died		
Average.....	12		12		19

The general effect with group 4, fed on sliced non-solanaceous foods exclusively and those members of group 1, which were fed on pepper plants or its fruit, was one of starvation. Some died after 7 days; others lived for 41 days but were unable to hiber-

<sup>1</sup> Potato beetles hibernating in July or November exhibit the same physiological reactions.

TABLE II.

NUMBER OF DAYS FOR BEETLES FED ON TUBERS AND FRUIT TO  
ENTER HIBERNATION.

Potato.		Tomato.		Eggplant.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
80.....	13	10.....	11	50.....	11
20.....	15	80.....	13	50.....	18
		10.....	Died		
Average....	14		12		14.5

*Cages Receiving No Water.*

80.....	11	10.....	11	10.....	11
20.....	13	90.....	18	90.....	13
Average....	12		14.5		12

TABLE III.

NUMBER OF DAYS FOR BEETLES FED ON FOLIAGE FOLLOWED BY SLICED TUBERS  
AND NON-SOLANACEOUS FOODS TO ENTER A STATE OF HIBERNATION.

Potato.		Squash.		Cucumbers.		Beets.		Carrots.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
75.....	15	80.....	25	40.....	15	40.....	13	40.....	15
25.....	25	20.....	35	50.....	21	50.....	38	10.....	38
				10.....	Died	10.....	Died	50.....	Died
Average..	20		30		18		25.5		25

TABLE IV.

BEETLES FED ON NON-SOLANACEOUS FOODS SHOWING NUMBER OF DAYS  
BEFORE DEATH.

Squash.		Cucumbers.		Beets.		Carrots.		No Food.	
Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.	Per Cent. Beetles.	Days.
40.....	37	40.....	15	60.....	37	40.....	15	100....	11
60.....	45	40.....	26	40.....	39	60.....	38		
		20.....	34						
Average..	41		25		38		24		

nate. In control cages where nothing was fed, the beetles died after 11 days.

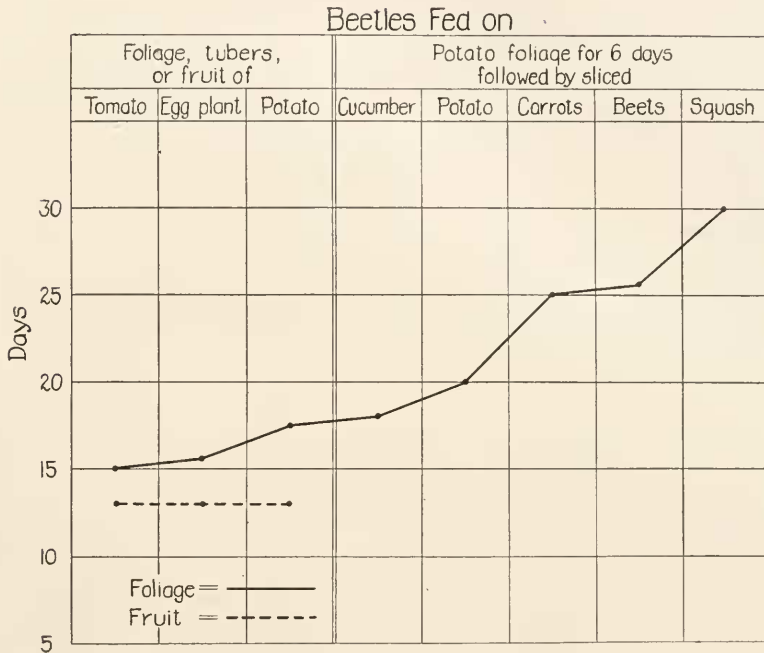


FIG. 2. Represents the number of feeding days required on different foods before the beetles entered a state of hibernation. Days are represented as ordinates, different foods as abscissa. The solid line curve shows the average number of days feeding on different foods before hibernation. The dotted line shows the average number of days feeding on tuber or fruit before hibernation.

In duplicate series of experiments conducted at the same time, certain cages received water to replace usual evaporation from the soil, others received no water. The addition of water to cages containing normal food plants decreased the feeding period from an average of 14.5 to 13.4 days. In control cages receiving only water the beetles died after 16 days. In cages containing non-solanaceous foods, the addition of water seemed to prolong the life of the beetles without inducing hibernation.

#### *Breeding Activities.*

It is perhaps essential to emphasize that potato beetles of the first generation used exclusively in these investigations indicated

no breeding activities before dormancy set in. The beetles hibernated during July and August and remained in that state until the following season. Other experiments conducted by confining first generation beetles with normal food plants in field cages, produced similar results. During fall and winter these hibernating beetles were frequently removed from the soil in the cages, and sections made of the testes revealed mature sperm in abundance. Dissections of the females yielded immature ova. These results are contrary to those obtained by Tower (24), who states, "The first brood on emergence feeds for a few days and then deposits eggs for a second generation. The second generation does not develop the germ cells nor show any reproductive activity until after it has passed through a period of hibernation or aestivation."

*Metabolism During Feeding Experiments.*

During the progress of feeding a veritable storing of food in the form of fat takes place in the adipose tissues of the animal (Fig. 4). It has been pointed out above that a numerical difference in days exists, for nutrient storage when different foods are fed. From metabolism determinations it seems possible to correlate the results procured during the feeding experiments with the oxygen consumption. In Fig. 3, the oxygen intake per gram organism in cubic millimeters per hour is shown. From an examination of this figure it is evident that a reduced oxygen consumption takes place when tuber or fruit material, as compared to foliage, is fed. Obviously then the process of converting tuber material required less oxygen. The analysis of food plants, given in Table V., shows the amounts of carbohydrate and fat

TABLE V.  
SHOWING ANALYSIS OF FOOD PLANTS IN PERCENTAGE.

Food Plant.	Water.	Protein.	Fat.	Carbo- hydrate.
Beets. ....	87.5	1.2	0.1	9.4
Carrots. ....	88.2	0.7	0.4	8.9
Cucumbers. ....	95.4	0.6	0.2	3.0
Eggplant. ....	92.9	0.9	0.3	4.9
Potato. ....	78.3	1.7	0.1	17.7
Squash. ....	88.3	1.1	0.5	8.6
Tomato. ....	94.3	0.7	0.4	3.8

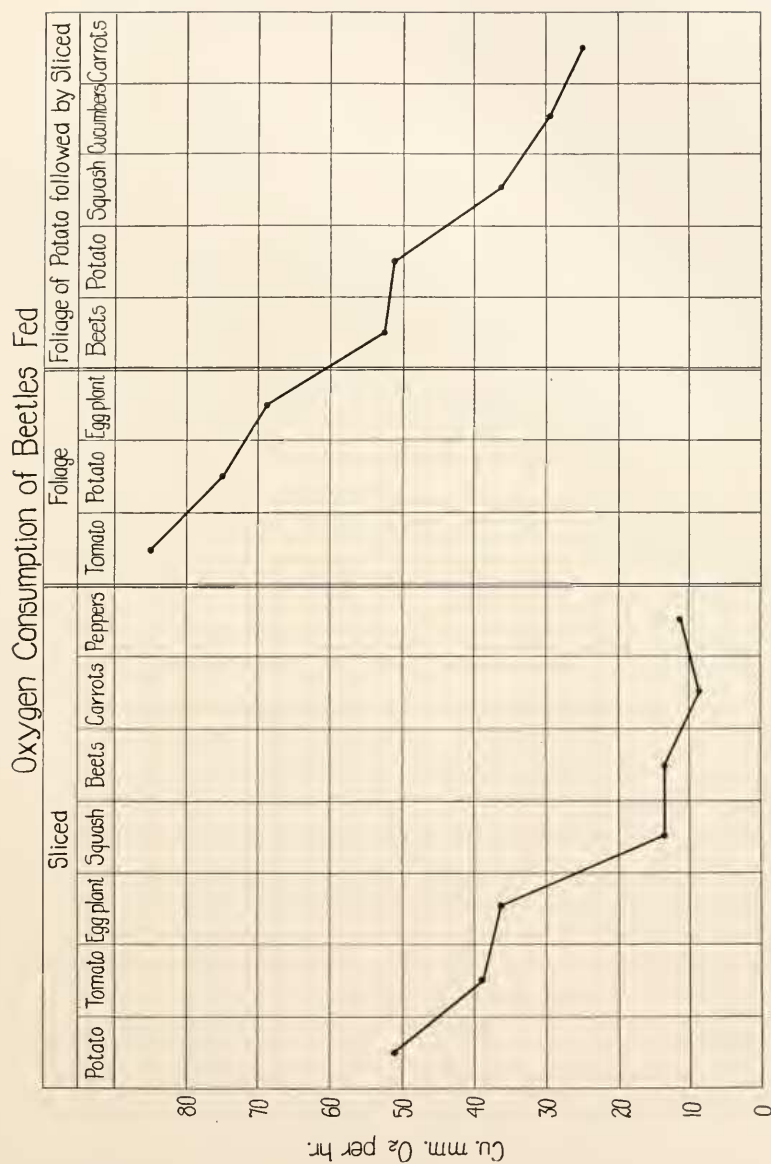


FIG. 3. Ordinates shows the oxygen intake in cubic millimeters per hour per gram body weight of beetles fed on different foods represented as abscissas. By comparison it is noted that distinctive differences in oxygen consumed are evident when potato beetles are fed tubers and fruit as compared to foliage of the same plants. This figure also shows the low oxygen intake when fed non-solanaceous foods.

present in non-solanaceous foods as compared to potato tuber and foliage. It is known that a diet rich in carbohydrates, as for example, turnips or potatoes, increases the proportion of lower fatty acids, while a diet rich in proteins diminishes this proportion. If the percentage of the lower fatty acids increases with the amount and kind of food, this fact may explain the results shown in the above experiments, since tuber material is rich in carbohydrates.

It was previously pointed out that members of group 4, fed non-solanaceous food plants and those beetles fed on peppers, in general, gradually starved. The respiratory metabolism of such animals manifests an extremely reduced oxygen consumption and (in other experiments to be discussed later) a decreased CO<sub>2</sub> output. In control experiments, where nothing was fed, the oxygen intake similarly was exceedingly diminished.

#### PREPARATION FOR HIBERNATION.

Of significance are the variations in weight recorded during the preparation for hibernation. First generation beetles as they emerged from pupation and before feeding averaged 0.1260 grams in weight. After feeding on foliage of the potato plant for ten days, the weight averaged 0.2627 grams. A state of quiescence or *prehibernation*<sup>2</sup> followed, varying from three to five days and was accompanied by a reduction of the water content and elimination of the waste material from the digestive tract, giving an average weight of 0.1879 grams. The increase in weight accounts, in part, for the accumulation of reserve nutrient material in the adipose tissues, and may, in addition, indicate growth of somatic and germinal cells. Furthermore, during *prehibernation* a gradual lowering of all vital activities takes place, the beetles afterwards entering the ground to hibernate.

Determinations of the water and fat content were made upon groups of beetles as shown in Fig. 4. From an examination of these it is evident that during active feeding, the average water and fat content was 76.4 and 7.8 per cent. respectively. During *prehibernation*, the average water and fat content was 59 and

<sup>2</sup> The term *prehibernation* is used to designate the quiescent state that occurs when feeding ceases and before the beetles burrow in the ground.



20 per cent. respectively. When the beetles burrowed in the ground the water content averaged 56 per cent. and the fat content 29 per cent. The results of these experiments indicate quite clearly that a loss of 20 per cent. in the water content and a gain of slightly over 20 per cent. in the fat content took place. Tower (24) obtained with this species a reduction of 27 per cent. in the water content. With hibernating grasshoppers Bodine (4) found a reduction of 13 per cent.

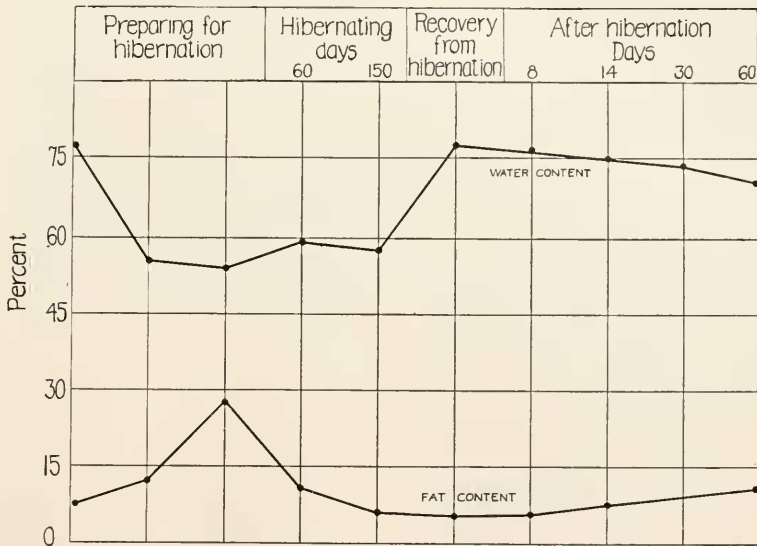


FIG. 4. Ordinates show the percentage of water and fat content of potato beetles, before, during and after hibernation. Note the reduction in the water content and increase in fat content as the beetles enter hibernation, and the extensive depletion in fat during the first few months as compared to the remaining period of hibernation.

The view commonly held is that a diminished water content makes protoplasm able to withstand extremes of temperatures. With potato beetles this does not appear to be valid, since invariably they must burrow in the ground to a considerable depth (10-18 inches) presumably to successfully escape extremes of temperatures. Experimentally the influence of greater variations in temperatures was determined by permitting only several inches of soil to intervene between the dormant beetles and the outside air. The results of such treatment upon hibernating

beetles during the winter, produced a mortality of 100 per cent. In this species a reduction of the water content probably does not proceed to a sufficient degree to prevent injury by freezing temperatures, hence necessitating their further protection by burrowing in the ground.

During the progress of hibernation, continued investigations have shown that the water content remains practically constant, although with a general diminution of fat, a slight increase in the water content may follow. Extremely significant is the excessive depletion in fat that obviously takes place during the first two months (51 per cent.) as compared to the remaining five months (49 per cent.) of hibernation.

In this connection it is of interest to cite, for comparison, the results of other investigators concerning fat consumption during hibernation. Victoroff (26) with frogs found a consumption of 23 per cent. Investigators with mammals demonstrated the prevalence of a large fat content which gradually disappears during the period of torpidity. Thus Voit (27) found that in the marmot the adipose tissue was more than 30 per cent. of the weight of the body. Valentin (25) found the adipose tissue in the marmot contributed about twenty times as much food as the hibernating gland. He also observed that the depletion in fat which occurred during the first few months was greater than after five months hibernation. Similarly Carlier (8) found that almost one half of the fat disappeared in the hedgehog during the first few months of dormancy. It is of interest to discover this general conformity between a mammal and an insect in the utilization of nutrient material during the course of hibernation.

#### *Hibernating Gland.*

Many investigators mention a hibernating gland which is said to be commonly found in mammals (hedgehog, marmot, bat, shrew, rat, mole, beaver, squirrel, weasel, martin, badger, rabbit, guinea pig, cat, dog and even in man). Rasmussen (18) who has reviewed the literature on this subject extensively, is of the opinion that it is distinct from adipose tissue. Sheldon (22) recently concluded that "the so-called hibernating gland is essentially a form of adipose tissue which retains its embryonic characteristics for a more or less indefinite length of time, . . .

it intergrades with ordinary adipose tissue and under favorable conditions is transformed into it."

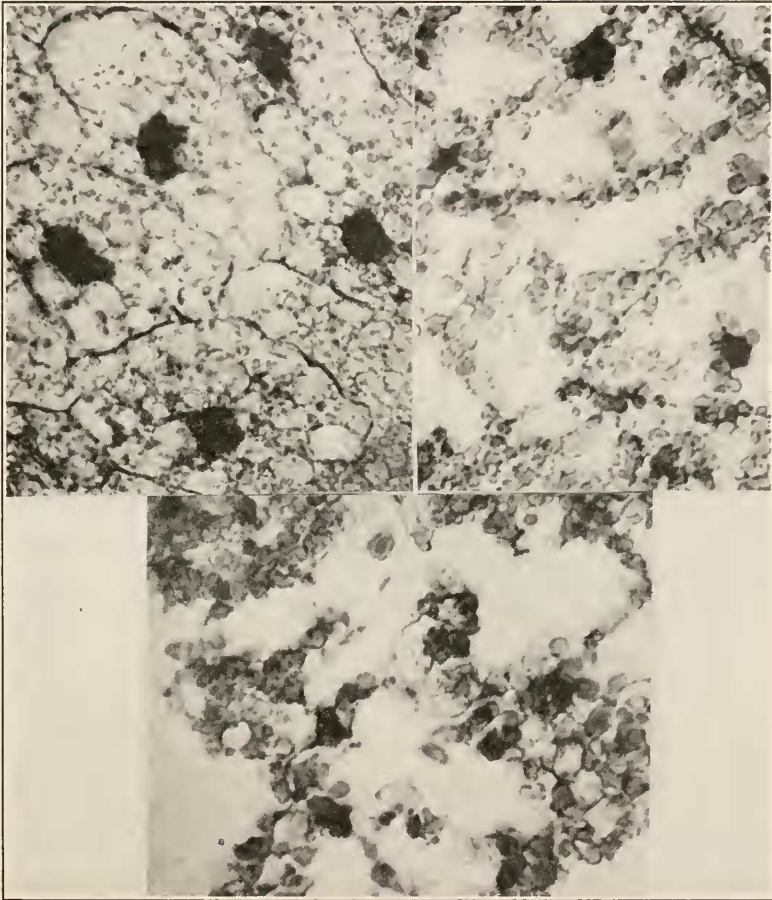


FIG. 5. Upper left. Section of the fat body or adipose tissue of potato beetle before hibernation. Note the numerous fat vacuoles partly masking the large nucleus located centrally within the cell, and the minute granules dispersed among the vacuoles. Flemming fixation, stained with hematoxylin. Photographed with achromat 2 mm., lens, 1.3 numerical aperture with 12X compensating eye piece.

FIG. 6. Upper right. Section of the fat body after one month of hibernation, showing the decreased size of the fat vacuoles and nucleus, the large and prominent albuminoid granules grouped along the periphery of the cell exhibiting open spaces. Staining and enlargement as in Fig. 5.

FIG. 7. Section of the fat body after five months hibernation, showing the almost complete absence of fat vacuoles. The prominently large albuminoid granules are grouped in masses, the absence of cell outline of the fat body and the almost entire disintegration of the nucleus. Staining and enlargement as in Fig. 5.

Whether it is ordinary adipose tissue or a special gland that functions in the mammal as a storage for nutrient material, with the potato beetle, and perhaps with insects in general, it seems quite certain that adipose tissue serves that purpose. To determine some of the morphological changes that take place during hibernation sections were made of the adipose tissues of the potato beetle. During *prehibernation* (Fig. 5) the sections show it to be composed of regular cells, oval in general outline and filled with conspicuous fat vacuoles of more or less uniform shape and size which extensively overlap and partly mask the actual contour of the large nucleus. Barely distinguishable are diminutive albuminoid granules dispersed among the vacuoles. The sections of beetles in hibernation for one month (Fig. 6) are strikingly different in appearance from those of non-hibernating ones. In the former we find that the fat vacuoles have become much smaller and that the nucleus has undergone disintegration and diminution in size. The most striking objects are the albuminoid granules aggregated along the periphery of the cells of the fat body, or clustered around the nucleus. The sections of adipose tissues of animals in hibernation for the longest period (six months) show clearly the prominently large albuminoid granules clustered in groups, exhibiting many open spaces (Fig. 7). Indications of a dissolution of the cells of the fat bodies and a dispersal of the albuminoid granules are quite evident.

The author compared Figs. 5-7, with those published by Rasmussen and Sheldon of sections taken from the hibernating gland in mammals, and the similarity is indeed very striking.

The existence of albuminoid granules or uric acid concretions indicates an active metabolism in the fat body. According to Lang (15) the fat body of larvae of insects is rich in fat and poor in concretions of uric acid before metamorphosis, while in the adult the opposite is true. Fabre (11) thinks that the adipose tissue serves the purpose of a urinary organ, since urates are formed within the cells. Both Graber (13) and Landois (14) regard it as a single many lobed lung (owing to the many fine branched tracheal endings in the fat body). Similarly Roubaud's view of the necessity of a physiological purification during

hibernation, based upon observing adipose tissue loaded with urate crystals before hibernation, must be regarded as signifying that it serves the purpose of an excretory organ.

There appears no valid reason for assigning to the fat body a urinary function. In the hibernating animal the metabolic activity of most cells is reduced to a minimum, and in the fat cells, judging from the respiratory quotient, it is most active. Hence the cells become charged with an accumulation of albuminoid granules and other products. The urates found in these cells are not an indication of their special urinary function, but of a more active metabolism at a period which serves the animal best. This may occur before or during metamorphosis, or throughout the progress of hibernation.

#### THE METABOLISM OF ACTIVE, STARVING AND HIBERNATING BEETLES.

A knowledge of the respiratory metabolism of active, starving and hibernating beetles seemed desirable for comparing the chemical changes involved. Of singular interest are the analogous rates of  $\text{CO}_2$  output procured from beetles emerging from hibernation and pupation (Figs. 8-9), indicating, perhaps, a condition of physiological youngness or purification (Roubaud) as a result of hibernation. Bodine (4) found hibernating grasshoppers produced a higher rate of  $\text{CO}_2$  output than growing animals kept at the same temperature, suggesting he states, "that animals remain young throughout the period of hibernation." The  $\text{CO}_2$  output of potato beetles throughout the progress of hibernation invariably indicates a reduced metabolic activity, in some respects comparable with that of starving animals (Fig. 9). Indeed, there appears to be a parallelism in respiratory metabolism between starving and hibernating forms. In the former, however, the velocity of reaction of the life processes continues to function most actively and reserve substances are rapidly depleted, a condition eventually leading to the death of the animal. In the latter most of the life activities are considerably depressed and nutrient material is, therefore, used sparingly.

With older beetles, the metabolic activities are also extremely reduced, but not to the extent met with in hibernating or starving

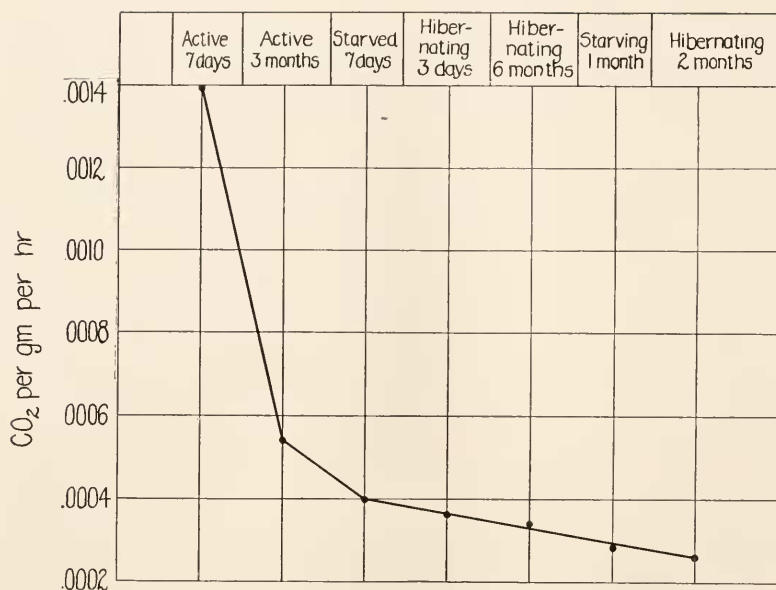


FIG. 8. Ordinates represent the CO<sub>2</sub> output per hour per gram weight of organisms. Note the reduced CO<sub>2</sub> output of hibernating and starving as compared to active animals, indicating a parallelism in metabolic activity between starving and hibernating beetles.

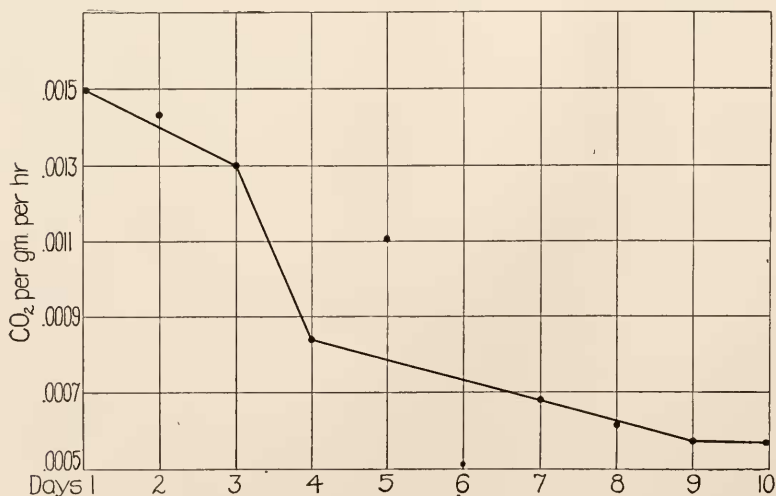


FIG. 9. Ordinates show the CO<sub>2</sub> output per hour per gram body weight of beetles during the progress of starvation until death. Abscissas represent days.



forms. The overfed and old state of the cell, as suggested by Baumberger (1) has perhaps reduced permeability to a wide degree. In old age or pathological conditions, according to Mathews (16), an accumulation of inactive ingredients or of acid in the cells, retards metabolic activity. In a measure, we are thus enabled to interpret the specific differences in metabolism that may take place under varying conditions in the organism. Especially noteworthy is the utilization of reserve nutrient material in the hibernating animal discussed further on.

#### *Respiratory Quotient.*

It is well known, experimentally, that when substances catabolized are chiefly fat, a reduced respiratory quotient results, whereas the catabolizing of carbohydrates and protein produces a higher quotient. According to the investigations of Dubois (10), Pembrey (17), Weinland and Riehl (29), Valentin (25) and others, reduced respiratory gas exchanges in the marmot occur during hibernation with strikingly low respiratory quotients (0.44-0.72). The views advanced to explain a quotient not consistent with the utilization of fat are many. Certain investigators (Dubois, Pembrey, Valentin) perceived that although mammals consumed no food during dormancy an increase in weight very often took place. It was, therefore, assumed that the oxygen consumed, greater than is necessary for fat oxidation, was in some way retained in the animal. Dubois also discusses building of acetone which he found accumulated in the blood-urine of hibernating mammals. It is known that of the normal fatty acids from butyric to decoic acid, only those with an even number of carbon atoms give rise to a marked increase in acetone formation. Dakin (9) and others have shown that acetone is derived from the decomposition of acetoacetic acid. This supports Knoop's theory of the B-oxidation in which two or some multiple of two carbon atoms are lost during oxidation, and offers a possible explanation that intermediate stages supervene throughout the process of the utilization of fat. Furthermore, Weinland and Riehl claim that during awakening from dormancy the marmot needs carbohydrates, and that the required carbohydrates come partly from substances produced

in the body during hibernation. But it is not certain whether they are produced from fat, protein, or both. Voit (27) holds that fat may form sugar, which in turn can be stored up as glycogen.

The respiratory quotients obtained with the potato beetle demonstrate a wide degree of variation during different periods of hibernation. The respiratory quotient is lowest during the

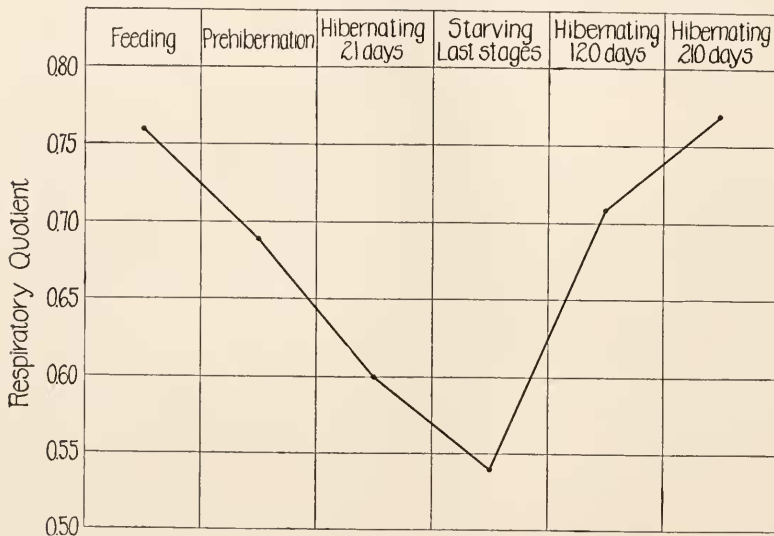


FIG. 10. Ordinates show the respiratory quotient based on respiratory metabolism ( $\text{CO}_2$  output and oxygen intake) of active beetles and of those after varying periods of hibernation as compared to starving animals.

first few months of dormancy and increases progressively. A noteworthy increase in the quotients results when the animals awakened to activity (Fig. 10). This may agree with the theory brought forward by Weinland and Riehl, that carbohydrate is being oxidized for the purpose of awakening. On the other hand, during acute stages of starvation, potato beetles evince an exceedingly reduced respiratory quotient (0.54). Dubois has shown that a hibernating mammal loses as much in weight in 160 days as a starving animal in 12 days. Since it is recognized that reserve nutrient materials are rapidly depleted during starvation it is possible, perhaps, to correlate the diminished respiratory quotient noted during the early phases of hibernation

with the immense decrease in the fat content which likewise results at this period.

#### RECOVERY FROM HIBERNATION.

In the preparation for hibernation the potato beetles pass through a quiescent phase which has been designated as *pre-hibernation*. Likewise on emergence from dormancy there is a period of quiescence known as recovery. From results obtained on respiratory metabolism during recovery and before feeding, it is evident that the increase in  $\text{CO}_2$  output is very gradual, lasting for six days (Fig. 11). The examples upon which the

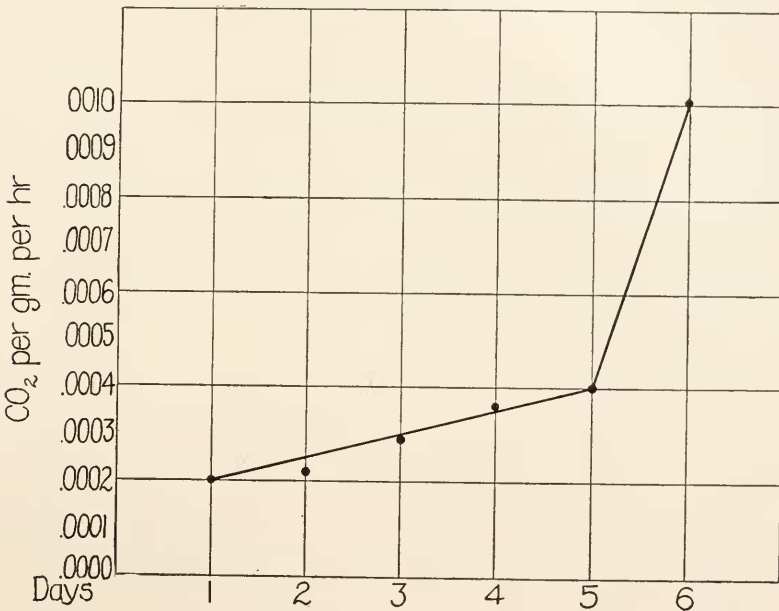


FIG. 11. Ordinates show the average  $\text{CO}_2$  output per hour per gram body weight of beetles during recovery from hibernation. This figure is based on group of beetles hibernating for six months.

above figure is based, comprised a group of beetles in hibernation for six months. After varying periods of dormancy, if potato beetles are removed from the ground and placed with food, we find they require from six to thirty days for recovery before feeding. Those that have been in hibernation for over two months require 30 days, those for three months 12 days, and

those five or more months 6 days. During the interval of restoration they are decidedly inactive, negatively heliotropic and do not feed.

Extensive experiments carried out during the winter with the view of hastening recovery proved fruitless. In every instance hibernating beetles when removed from the ground and placed with food persistently indicated tendencies to burrow themselves in again. In those cages not provided with soil the beetles remained inactive on the side or bottom of the cage without feeding.

Both Bodine (4) and Sanderson (20) found that the rate of recovery was quickest with animals brought in later in the hibernating period. Sanderson, however, concluded that subjecting hibernating forms to low temperature had the effect of producing a more complete rest than at a higher temperature. In view of additional experiments performed by the author a different interpretation may be attributed to the above results. For example, beetles reared in the greenhouse hibernated the latter part of March and were kept at a comparatively high temperature (65–95° F.) in the greenhouse. On July 11, they were found issuing from the ground in the cages, exhibiting normal activities such as feeding, mating, etc. In these experiments, therefore, cold was not essential for a complete rest. Furthermore, the depth at which potato beetles normally hibernate in the soil (10–18 inches) precludes the assumption of a specific influence of low temperature, or of the necessity of subjecting them to prolonged periods of cold. On the contrary, experimental evidence demonstrates a rhythmical period of rest irrespective of temperature.

#### *Adjustment of the Water Content.*

Of paramount importance to the animal during recovery and before feeding is possible, is an adjustment of its water content to normal. The need for actual contact of the animal with water at different intervals seems to be essential. Whether moisture may also be absorbed through the integument is not definitely known.

In the following experiments beetles recovering from hibernation were kept in cages with abundance of food under the

same temperature conditions in the greenhouse. One lot frequently received water, the other lot was not watered. The addition of water (a drenching of the cage) had an activating effect, since the beetles eventually became active and fed normally on the plants. In those cages not receiving water the beetles were apparently unable to recover and finally perished. The lack of actual contact of the animal with water evidently hindered recovery.

Dissected hibernating beetles invariably revealed accumulated waste products in the rectum. In normal animals its expulsion is necessary before activity and feeding is possible. It seems reasonable that water actually is imbibed by the animal during recovery to aid in the elimination of inert substances from the digestive tract.

#### CATALASE AND OXIDASE ACTIVITY.

It seemed desirable to determine if other factors concomitant with a lowered metabolic activity are involved in the hibernating animal. There are many facts in the literature tending to show that the power of decomposing hydrogen peroxide and the power to blue guaiacum by the aid of peroxide are the specific property of certain substances. Since the recognition of catalase as a specific enzyme, its occurrence and distribution in various animal and vegetable tissues has been investigated by a number of observers.

Certain investigators are of the opinion that the accumulation of hydrogen peroxide would undoubtedly prove harmful to the organism, and that the function of the catalase is to destroy the hydrogen peroxide as fast as it is formed. Others hold that the function of catalase may be to prevent the excessive oxidation of organic substances in the living cell. Schoenbein (21), however, has shown that substances which can bring about the decomposition of hydrogen peroxide catalytically, can also greatly increase its oxidizing power, and in proportion as a substance is able to decompose the peroxide so also it can accelerate oxidation. The power to decompose hydrogen peroxide is held by Spitzer (23) to be a measure of the oxidizing power of various animal tissues. Dakin (9), however, is of the opinion

that there is no trace of evidence to prove that catalase is directly concerned with oxidation, since catalase, he states, "only liberates inactive molecular oxygen when decomposing hydrogen peroxide." Burge (7) found a quantitative relation between the amount of catalase and oxidation; an increase of oxidation in young and a decrease in old animals. Similarly Bodine (5) with certain insects obtained a decreased catalase content with increasing age and with animals subjected to starvation.

The author made determinations of the activity and total content of catalase in hibernating, starving, young and old potato beetles, using for this purpose the same methods described by Burge. The results of these experiments are graphically represented in Figs. 12-13. With hibernating animals the total catalase content per gram body weight is lower than with either

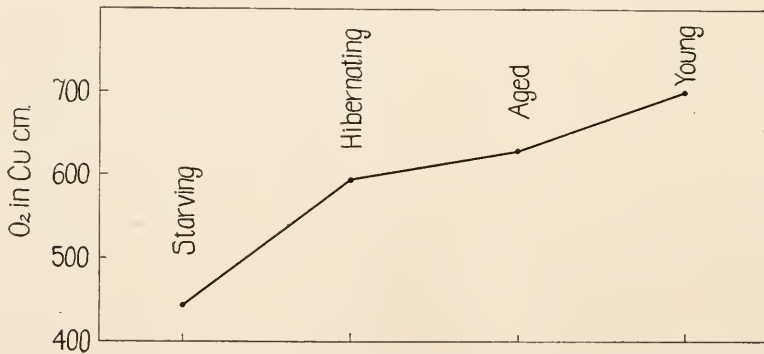


FIG. 12. The average total catalase content per gram body weight of different groups of beetles as measured by the oxygen evolved. Ordinates show the oxygen evolved in cubic centimeters.

young or old beetles; starving animals indicate the lowest catalase content. In Fig. 13, the catalase activity per gram body weight per minute is recorded and strikingly indicates a seeming parallelism in catalase activity between hibernating and starving animals. Moreover, the extremely reduced catalase activity indicates a correlation with a diminished respiratory metabolism in hibernating and starving animals.

Experiments to determine the oxidase activity were made by using guaiac, P-diamino benzene with peroxide upon tissues, organs and body fluids. Upon hibernating animals the reaction



were negative since no typical characteristic blueing of guaiacum took place. Similar tests performed with active and starving beetles, gave striking reactions (blueing of guaiacum) with esophagus, stomach, tip of rectum, tissues, body fluids, testes and immature ova.

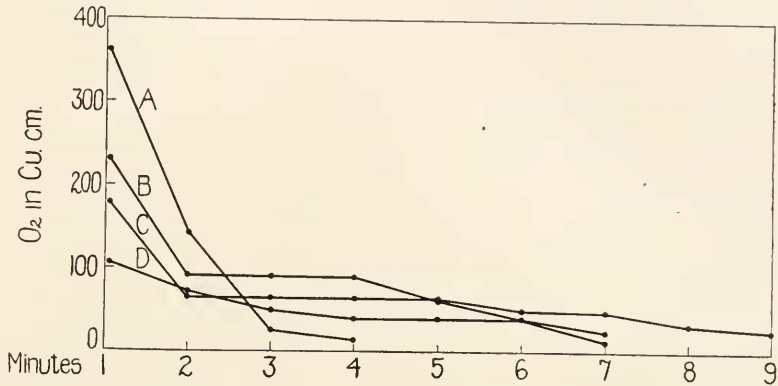


FIG. 13. The catalase activity per minute per gram body weight of organism. Ordinates show the oxygen evolved in cubic centimeters, abscissas as minutes. A, young beetles; B, aged beetles; C, hibernating beetles; D, starving beetles.

Although the catalases and oxidases may possess specific functions in the active animal, in the hibernating forms these enzymic activities obviously become greatly reduced or seemingly absent.

#### SUMMARY.

1. The results of the food experiments indicate that potato beetles of the first generation when fed on tubers and fruit of the potato, tomato, and eggplant require 13 days feeding to enter hibernation, while those fed on foliage of the same plants need 16 days. Beetles fed on potato foliage for six days followed by a non-solanaceous food required 23.6 days feeding before hibernation, and produced a mortality of 55 per cent. Those fed on pepper plants or on non-solanaceous foods do not hibernate, but die of starvation.

2. Respiratory metabolism determinations show that the oxygen consumption of beetles fed on tubers or fruit of solanaceous plants is less than of those fed on foliage. When fed on non-solanaceous foods, the reduced oxygen intake recorded was

comparable with the oxygen consumption of starving animals. A reduced  $\text{CO}_2$  output occurs throughout the progress of hibernation.

3. Preparation for hibernation follows a period of extensive feeding and consists in an accumulation in the adipose tissues of 29 per cent. fat. During prehibernation a reduction of the water content of 20 per cent., the elimination of waste products from the digestive tract and a lowered metabolic activity of the animal take place.

4. The greatest depletion in fat occurs during the first few months of hibernation. In the metabolism of the fat body, the fat is replaced by albuminoid granules and other products. Sections of adipose tissue before and after hibernation are comparable to sections of the hibernating gland of mammals.

5. The respiratory quotient is lowest during the first few months of hibernation and highest at the termination of dormancy (0.60-0.76). In hibernating animals possessing insufficient nutriment and in starving animals, the respiratory quotient is exceedingly low (0.54). There appears to be a parallelism between a rapid depletion of reserve food and a low quotient.

6. Recovery depends upon the length of time potato beetles spend in hibernation. It is more rapid with those in hibernation for the longest time. Actual contact of the animal with water seems necessary to restore its water content before feeding is possible.

7. Catalase activity of hibernating beetles is greatly reduced and their total catalase content is lower than that for old or young active beetles. There appears to be a correlation between the reduced catalase activity and diminished respiratory metabolism in hibernating and starving animals.

Although active beetles give very striking oxidase reactions, no characteristic blueing of guaiacum was obtained with any organ, tissue, or body fluid of hibernating forms.

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